On the Origin of Oxygenic Photosynthesis and Cyanobacteria

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Summary

Oxygenic phototrophs have played a fundamental role in Earth's history by enabling the rise of atmospheric oxygen (O_2) and paving the way for animal evolution. Understanding the origin of oxygenic photosynthesis and Cyanobacteria are key when piecing together the events around Earth's oxygenation. It is likely that photosynthesis evolved within bacterial lineages that are not extant, so it can be challenging when studying the early history of photosynthesis. Recent genomic and molecular evolution studies have transformed our understanding about the evolution of photosynthetic reaction centres and the evolution of Cyanobacteria. The evidence reviewed here highlights some of the most recent advances on the origin of photosynthesis both at the genomic and gene family level.

Introduction

I. What is the evidence for the origin of oxygenic photosynthesis and Cyanobacteria?

Oxygenic photosynthesis is one of the most important metabolisms to have evolved on Earth as it enabled complex life to emerge. A fundamental question in biology is when oxygenic photosynthesis first evolved. It underpinned both biological and geological processes that led to the rise of O_2 during the Early Earth. Regarding the timing of origin, the fossil record of Cyanobacteria is not conclusive (Schirrmeister *et al.*, 2016). Similar fossil evidence has shown that molecular biomarkers are no longer reliable (Rasmussen *et al.*, 2008).

Different lines of geochemical evidence have constrained the major oxygenation events observed during Early Earth. The first rise of oxygen in the atmosphere, known as the Great Oxidation Event (GOE) ~ 2.32-2.4 Billion years ago (Bya) (Bekker *et al.*, 2004; Lyons *et al.*, 2014), has been well constrained based on geochemical proxies (Lyons *et al.*, 2014). The GOE represents the minimum age for the origin of oxygenic photosynthesis and the emergence and diversification of oxygen-requiring metabolic and biosynthetic pathways (Raymond & Segre, 2006). A second major oxygenation event known as the Neoproterozoic Oxidation Event (NOE) ~800-600 Million years ago (Mya), significantly increased atmospheric O_2 concentrations (Scott *et al.*, 2008) similar to those found in today's atmosphere. The NOE has

also been linked to the origin of animals, major glaciation events and huge disruptions to the carbon cycle (Lyons *et al.*, 2014), and more recently to the emergence of marine planktonic groups (Brocks *et al*., 2017). Over geological time, O_2 in our atmosphere has accumulated because carbon fixation by oxygenic phototrophs has exceeded respiration of organic matter. Simultaneously, the imbalance of these two processes has resulted in the drawdown of carbon and burial of organic carbon in marine sediments (Holland, 2006).

While it is well recognised that O_2 has been the result of biological activity contributing to the oxidation events during the Precambrian (Lyons *et al.*, 2014), less is known about the evolutionary history of oxygenic phototrophs. How did their origination and subsequent diversification into different functional groups and/or taxa contribute to shaping geological processes? Clues about the evolution of early oxygenic phototrophs have been recorded in their genomes, and their history can be elucidated through phylogenetic comparison and comparative genomics. The evidence reviewed here focuses on the biological evidence, partly because over the last decade, the increased number of genomic studies have transformed our understanding on the evolution of photosynthesis and oxygenic phototrophs (Blank & Sánchez-Baracaldo, 2010; Soo *et al.*, 2014; Sánchez-Baracaldo *et al.*, 2017).

II. Timing of divergence of oxygenic photosynthesis and major Cyanobacteria groups

Genomics and evolutionary studies have provided insights into the evolution of core proteins involved in oxygenic photosynthesis (Cardona, 2018; Cardona *et al.*, 2019) and the appearance of Cyanobacteria's common ancestor (Blank & Sánchez-Baracaldo, 2010; Schirrmeister *et al.*, 2013; Shih *et al.*, 2016). Amongst prokaryotes, Cyanobacteria have some of the best fossil records (Schirrmeister *et al.*, 2016) enabling molecular clock studies. Ages estimates of the gene family of PSI and PSII (Cardona, 2018; Cardona *et al.*, 2019) are consistent with geological records showing traces of oxygen throughout the Archean (4–2.5 Bya); these findings imply that oxygenic photosynthesis was already established by 3.0 Bya *et al.*, 2014; Wang *et al.*, 2018). In other words, early forms of water oxidation, carried out by ancestral homodimeric photosystems (Figures 1 and 2), could have originated a billion years before the GOE (Cardona *et al.*, 2019). Furthermore, the standard heterodimeric photosystems, a defining trait of crown group Cyanobacteria (Figure 3), evolved towards the late Archean (Blank &

Sánchez-Baracaldo, 2010; Schirrmeister *et al.*, 2015) or early Paleoproterozoic (Shih *et al.*, 2016).

The majority of extant Cyanobacterial diversity evolved after the GOE (Figure 3) (Sánchez-Baracaldo, 2015). For instance, the closest relatives (i.e., *Gloeomargarita*) of the Archaeplastida, a monophyletic group that includes the glaucophytes, red algae, the green algae and land plants emerged ~1.9 billion years ago (Sánchez-Baracaldo *et al.*, 2017). In more recent time scales, the age estimation of marine planktonic groups is consistent with geochemical evidence supporting the timing of the ocean oxygenation at around 800–600 Mya (Sánchez-Baracaldo *et al.*, 2014). Age estimates of marine green algae (Sánchez-Baracaldo *et al.*, 2017) at the end of the Precambrian and prior to the origin of animals are consistent with eukaryote biomarker data (Brocks *et al*., 2017). Molecular clock studies of symbiotic associations have also shown that age estimates of the symbiont, UCYN-A, overlap with fossil ages of its host, *Braarudosphaera bigelowii*, at around 92 Mya (Cornejo-Castillo *et al.*, 2016).

III. The origin of Photosystem II (PSII) vs the origin of Cyanobacteria

Photosynthesis is an ancient metabolism that likely evolved in lineages that are no longer extant. Today photosynthetic reaction centres are found amongst at least eight extant bacterial lineages: Cyanobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Chlorobi, Firmicutes, Gemmatimonadetes, and the newly discovered Candidatus Eremiobacterota (Figure 1) (Hug *et al.*, 2016; Ward *et al.*, 2019). Since oxygenic photosynthesis is only found in Cyanobacteria, and other groups of bacteria evolved different types of anoxygenic photosynthesis (Hohmann-Marriott & Blankenship, 2011), it is often assumed that the appearance of oxygenic photosynthesis coincided with the origin of Cyanobacteria (Soo *et al.*, 2017). While to an extent, it is reasonable to interchange both terms, there are significant differences when referring to oxygenic photosynthesis and Cyanobacteria. At the gene family level, the origin of reaction centre proteins elucidate the origin of photosynthetic water oxidation (Cardona, 2018; Cardona *et al.*, 2019). At the organismal level, phylogenomic approaches unravel the evolutionary history of organisms that are currently able to perform oxygenic photosynthesis (Blank & Sánchez-Baracaldo, 2010; Schirrmeister *et al.*, 2013; Schirrmeister *et al.*, 2016). Both approaches have helped piece together biological events that have been obscured by over 3 billion years of history.

It has been widely assumed that oxygenic photosynthesis emerged from ancestral anoxygenic phototrophs (Hohmann-Marriott & Blankenship 2011). Cyanobacteria have two photosystems (PSI and PSII), and anoxygenic phototrophs have either PSI or PSII-like photosystems. Some have proposed that a "protocyanobacteria" containing two anoxygenic photosystems predated Cyanobacteria from an ancient duplication (Mulkidjanian *et al.*, 2006; Martin *et al.*, 2018), whereas others have put more emphasis on whether the photosystems emerged by horizontal gene transfers (Raymond *et al*., 2002). Recent work bringing together comparative structural biology and phylogenetic analyses have challenged some of these older perspectives. It is now argued that the photosystems (marked 1 in Figure 1) uniquely evolved the ability to perform water oxidation from the beginning, and as the photosystems further specialised, it led to what we now know as oxygenic photosynthesis (Cardona, 2017; Cardona, 2019; Cardona & Rutherford, 2019). It is worth highlighting that the efficiency of enzymes carrying out water oxidation has changed and improved, and earlier forms predate the evolution of crown group Cyanobacteria (Cardona et al., 2019) (marked 5 in Figure 1, and Figure 3).

Large scale phylogenetic analyses have confirmed that photosynthetic organisms are polyphyletic, or do not share a recent common ancestor (Cardona, 2015; Hug *et al.*, 2016). In other words, lineages of phototrophs are often closely related to non-photosynthetic lineages. This is the case for Cyanobacteria, in which their sister groups are non-photosynthetic such as the Melainabacteria (Di Rienzi *et al.*, 2013), and the Sericytochromatia (Soo *et al.*, 2017). Melainabacteria and Sericytochromatia lack genes involved in photosynthesis (Soo *et al.*, 2017) and likely lost the ability to perform photosynthesis after their divergence from Cyanobacteria. Phylogenetic and comparative analyses further indicate that the most recent common ancestor of Cyanobacteria was already a highly sophisticated phototroph capable of water oxidation (Blank & Sánchez-Baracaldo, 2010; Cardona *et al.*, 2015).

IV. PSII

The core of cyanobacterial PSII consists of D1 and D2 (Figure 2), which originated from an ancient gene duplication event (marked 4 in Figure 1). These proteins are associated with two core antenna subunits named CP43 and CP47 (Figure 2), which also originated from an ancient gene duplication event. The origin of water oxidation likely predated these two duplication events (Rutherford & Faller, 2003; Cardona *et al.*, 2019). Biochemical evidence implies that

the ancestral homodimeric PSII (marked 4 in Figure 1) was a highly-oxidising, oxygen-producing photosystem that had already evolved the capacity to protect against the formation of reactive oxygen species (Cardona *et al.*, 2019).

In-depth analysis of the rates of evolution of PSII suggest that the gene duplication associated with evolution of D1 and D2 (marked 4 in Figure 1) predated the most recent common ancestor of Cyanobacteria (marked 5 in Figure 1) (Cardona et al., 2019). PSII is the slowest evolving photosystem, displaying rates of evolution up to five times slower than those of anoxygenic photosystems. This means that the earliest Type II reaction centres (marked 2 in Figure 1), previously thought to be similar to those present in purple bacteria (Proteobacteria, marked 6 in Figure 1), were more like cyanobacterial PSII. When comparing the overall structural architecture of the photosystems (Figure 2), it emerges that cyanobacterial PSII retains a greater number of ancestral traits than the reaction centres of Proteobacteria and Chloroflexi (Cardona & Rutherford 2019). It is worth highlighting that the deep divergence of anoxygenic and oxygenic photosystems (marked 2 and 3 in Figure 1), could have been a response to dealing with oxygen itself (Orf et al., 2018; Cardona, 2019). In other words, the origin of water oxidation to oxygen not only predated the D1 and D2 duplication (marked 4 in Figure 1), but may have coincided with the emergence of the two distinct families of reaction centres themselves (marked 1 in Figure 1) (Cardona & Rutherford, 2019).

V. Crown group Cyanobacteria

Within prokaryotic groups, Cyanobacteria are one of the most morphologically diverse - growth forms range from unicellular to filamentous or multicellular (Castenholz, 2001; Shih *et al.*, 2013). Not long ago, most of the available genome data for Cyanobacteria were biased toward marine unicellular taxa (e.g., *Synechococcus* and *Prochlorococcus*) (Shih *et al.*, 2013). Within the last five years, a number of studies have isolated and sequenced lineages covering a wider range of taxonomic diversity and habitats within the tree of life of Cyanobacteria. Some of these studies have included: *Gloeomargarita* also known as the closest known relative of the chloroplast (Ponce-Toledo *et al.*, 2017; Sánchez-Baracaldo *et al.*, 2017); *Pseudanabaena* (Schirrmeister *et al.*, 2015b); symbiotic groups from both marine and freshwater habitats (e.g., UCYN-A, *Richelia, Epithemia, Rhopalodia*) (Hilton *et al.*, 2013; Bombar *et al.*, 2014; Nakayama *et al.*, 2014; Cornejo-Castillo *et al.*, 2016); extremophiles from cold extreme habitats (Chrismas *et al.*, 2016; Chrismas *et al.*, 2018); underrepresented freshwater genomes

(Di Cesare *et al.*, 2018; Sánchez-Baracaldo *et al.*, 2019); and genomes from continental subsurfaces (Puente-Sánchez *et al.*, 2018).

The availability of new genomes and large-scale phylogenetic analyses have helped resolve deep-branching relationships within Cyanobacteria, providing insights into the evolution of morphology and habitat within this Phylum (Blank & Sánchez-Baracaldo, 2010; Shih *et al.*, 2013; Schirrmeister *et al.*, 2015). Genomic data combined with advances in phylogenetic and trait evolution analyses have filled gaps in the geological record by providing testable hypotheses about the ancestral habitat of ancestral Cyanobacteria (Blank & Sánchez-Baracaldo, 2010; Schirrmeister *et al.*, 2016; Sánchez-Baracaldo *et al.*, 2017).

Trait evolution analyses have shown that early divergent Cyanobacteria likely inhabited low salinity and terrestrial environments (Blank & Sánchez-Baracaldo, 2010). The earliest Cyanobacteria forms were unicellular and had small cell diameters (*Gloeobacter*, *Synechococcu*s-like) (Larsson *et al.*, 2011; Sánchez-Baracaldo, 2015). Filamentous forms appeared shortly afterwards and likely resembled extant *Pseudanabaena* lineages (Figure 3) (Schirrmeister *et al.*, 2011; Sánchez-Baracaldo, 2015). Their emergence would have facilitated the formation of microbial mats increasing their ecological dominance during the Proterozoic (Sánchez-Baracaldo, 2015; Schirrmeister *et al.*, 2016). The origin of multicellularity in Cyanobacteria was a significant biological innovation that has been previously associated with increased diversification rates around the GOE (Schirrmeister *et al.*, 2013) resulting in most of the diversity of extant Cyanobacteria, including recently described groups such as Macrocyanobacteria (cell diameters larger than 3 μm up to 50 μm) and Microcyanobacteria (cell diameters ranging from ~ 1-2 μm) (Sánchez-Baracaldo, 2015).

The great majority of extant Cyanobacteria are found in terrestrial and freshwater environments, and often thrive as pioneer species in habitats such as drylands, glaciers and the open ocean (Castenholz, 2001; Blank & Sánchez-Baracaldo, 2010). Phylogenomic analyses have further revealed that marine planktonic lineages are derived taxa (Sánchez-Baracaldo *et al.*, 2014). Some of these lineages are sister to unicellular freshwater taxa (e.g., *Synechococcu*s, and *Cyanothece*), filamentous freshwater (e.g., Nostocales) and benthic marine mat formers (e.g., *Hydrocoleum*) (Sánchez-Baracaldo *et al.*, 2014; Sánchez-Baracaldo, 2015). In other words, marine planktonic lineages do not form a monophyletic group; this phylogenetic pattern provides evidence for independent colonization events into open ocean

habitats at different times in history (Sánchez-Baracaldo *et al.*, 2014; Sánchez-Baracaldo, 2015; Cornejo-Castillo *et al.*, 2016; Sánchez-Baracaldo *et al.*, 2019).

VI. Conclusions and Future Perspectives

Recently available genomic data and advancements in evolutionary methodologies have helped to resolve our understanding of the evolution of photosynthetic reaction centres and Cyanobacteria. Biological evidence supports the view that early forms of oxygenic photosynthesis were present throughout the Archean. Evolutionary studies of PSII imply that oxygenic photosynthesis was already well established by 3.0 Bya reconciling geochemical and molecular evolution evidence bases. Consequently, crown group Cyanobacteria may have become the dominant primary producers near the late Archean as oxygenic photosynthesis reached a higher level of complexity and sophistication. Most major groups of Cyanobacterial diversity, including the lineage leading to chloroplasts, appeared after the GOE. Marine planktonic groups evolved toward the end of the Precambrian when biomarker and molecular clock analyses point to the first appearance of marine eukaryotic green algae prior to the emergence of animals.

Some outstanding question remain regarding the evolution of photosynthesis. Further research is required to fully determine the structural and photochemical characteristics of the earliest known reaction centres and to identify the evolutionary incentives behind establishment of two photosystems in series. This could be accomplished with ancestral sequence reconstruction strategies. Future efforts should also continue uncovering diversity from early divergent lineages. Close relatives of Cyanobacteria, such as Melainabacteria and Sericytochromatia, have been identified almost entirely from metagenomic data. It is therefore important to isolate and culture these lineages of non-photosynthetic close relatives to explore their metabolic and physiological capabilities. Other basal lineages such as *Gloeomargarita* (Ponce-Toledo *et al.*, 2017), have revealed the closest known relatives of the chloroplast and helped to infer the habitat of early photosynthetic eukaryotes (Sánchez-Baracaldo *et al.*, 2017). Finally, age estimates could be improved by implementing calibration points, including new taxa and experimentally measuring rates of cyanobacterial genome evolution across taxa.

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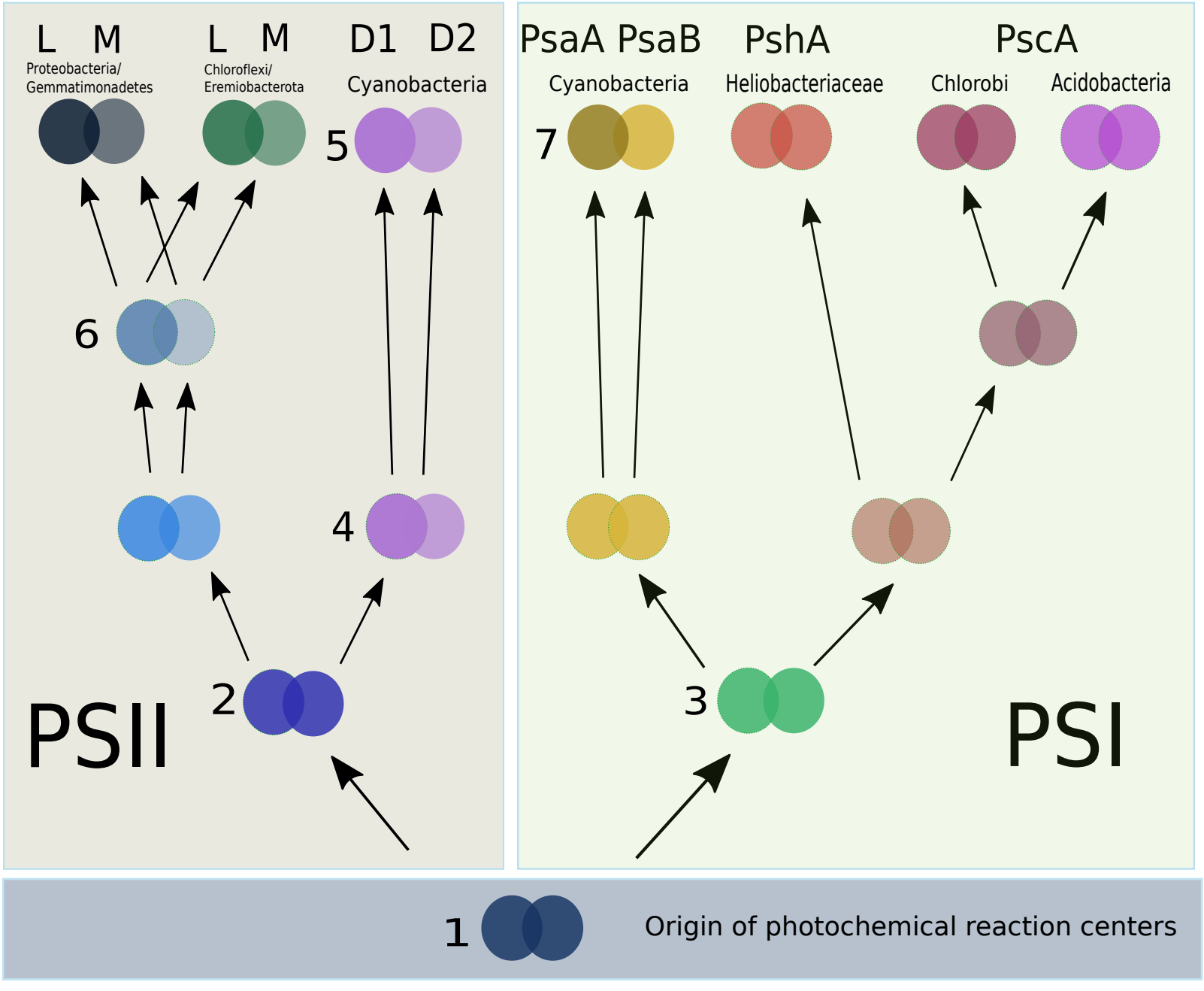
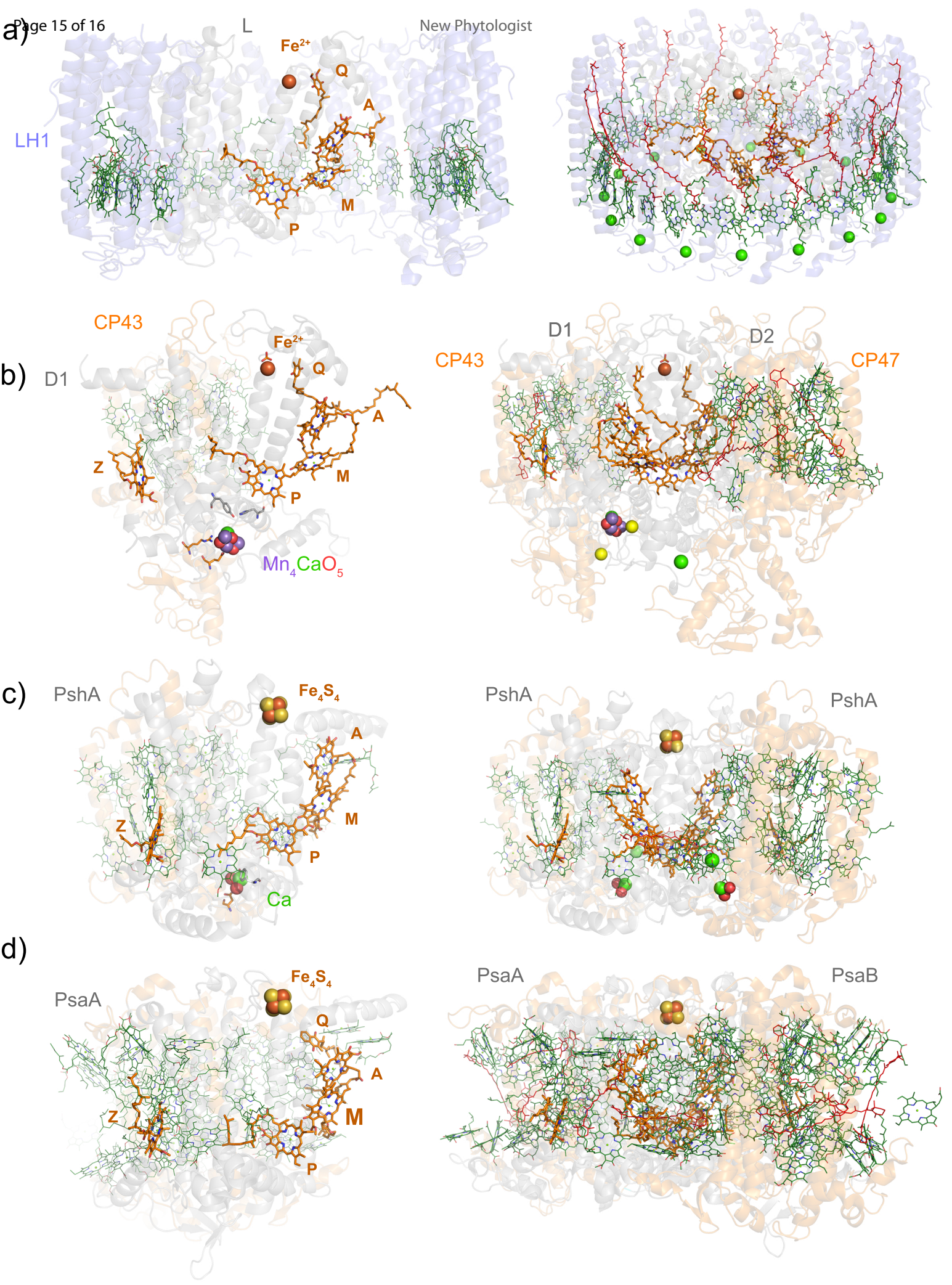
Figure 1. Schematic representation of the evolutionary relationships of reaction centre proteins based on molecular phylogenies. The ancestral photochemical reaction centre protein was likely encoded by a single gene (bottom, marked 1), which later gave rise to the first PSII-like (Type II) and PSI-like (Type I) reaction centre proteins. The monophyly of all reaction centre proteins (marked 2 and 3) implies that their origin predates the radiation of all known groups of phototrophs. At the beginning, both ancestral types were homodimeric (indicated by the same colour tone). Type II reaction centres later became heterodimeric (indicated by different colour tones) by convergent evolution in cyanobacterial PSII (marked 4) and in anoxygenic Type II reaction centres (marked 6). Crown group Cyanobacteria inherited heterodimeric PSII (marked 5) and heterodimeric PSI (marked 7).

Figure 2. Structural comparisons of anoxygenic and oxygenic photosystem cores. Monomers displaying the main redox cofactors, molecules in orange sticks, are shown in the left column and full dimeric configurations are shown in the right column. Transparent grey ribbons mark the core subunit associated with the core antenna displayed in transparent orange ribbons. Antenna (bacterio)chlorophylls are shown in green lines, with the exception of that marked as Z; carotenoids are shown in red lines. Type II reaction centres can be visually recognised by the presence of a non-heme Fe^{2+}, while Type I reaction centres feature an iron-sulfur cluster, F_X. a) Anoxygenic Type II reaction centre of the purple bacteria (phototrophic Proteobacteria). Only the reaction centre core subunit L is shown surrounded by the light harvesting complex LH1 (purple ribbons). b) Oxygenic cyanobacterial PSII. The core of PSII is comprised of the reaction centre subunits D1 and D2, and the core antenna subunits CP43 and CP47. c) Homodimeric Type I reaction centre of the Heliobacteria (phototrophic Firmicutes). The core of this contains a single subunit known as PshA. d) Cyanobacterial heterodimeric PSI. The core of PSI is comprised of two subunits known as PsaA and PsaB. P (photochemical pigment), M (monomeric chlorophylls), A (primary acceptor), and Q (quinone) mark the different redox cofactors at homologous positions between different photosystems. Z denotes a core-bound antenna chlorophyll retained in PSII and Type I reaction centres but lost in the anoxygenic Type II reaction centres concomitant with the loss of core antenna and the evolution of a new light harvesting system.

Figure 3. Timeline of the emergence of PSI, PSII and Cyanobacterial lineages. Age estimates for PSI (Cardona, 2018), PSII (Cardona *et al.*, 2019), crown group Cyanobacteria (Schirrmeister *et al.*, 2015b), major clades and taxa (Sánchez-Baracaldo, 2015; Sánchez-Baracaldo *et al.*, 2017). The timing of the Great Oxidation Event (GOE) (Bekker *et al.*, 2004), Gunflint formation (Fralick *et al.*, 2011) and Neoproterozoic Oxidation Event (Och & Shields-Zhou, 2012). Cartoons are not drawn according to scale. Ancestral forms of oxygenic photosynthesis powered by homodimeric PSII and PSI emerged in the early Archean or early Paleoproterozoic. D0 denotes an ancestral core subunit before the gene duplication that led to D1 and D2; it is thought to have assembled into a primordial water-splitting photosystem. The most recent common ancestor of Cyanobacteria inherited a heterodimeric photosystem shared by all extant oxygenic phototrophs. Taxa with smaller cell diameter (basal lineages and Microcyanobacteria) are shown at the bottom and larger cell diameter (Macrocyanobacteria) at the top. Major Cyanobacterial clades radiated into many diverse forms after the GOE. Marine planktonic Cyanobacteria evolved towards the end of the Precambrian, and the Cretaceous.


L M
Proteobacteria/
Gemmatimonadetes
L M
Chloroflexi/
Eremiobacterota
D1 D2
Cyanobacteria
5
6
4
2
PSII
PsaA PsaB
Cyanobacteria
PshA
Heliobacteriaceae
PscA
Chlorobi
Acidobacteria
7
3
PSI
1
Origin of photochemical reaction centers


a)
L
LH1
Fe2+
Q
A
M
P
b)
CP43
D1
Fe2+
Q
A
Z
M
P
Mn4CaO5
D1
D2
CP43
CP47
c)
PshA
Fe4S4
A
Z
M
P
Ca
PshA
PshA
d)
PsaA
Fe4S4
Q
A
Z
M
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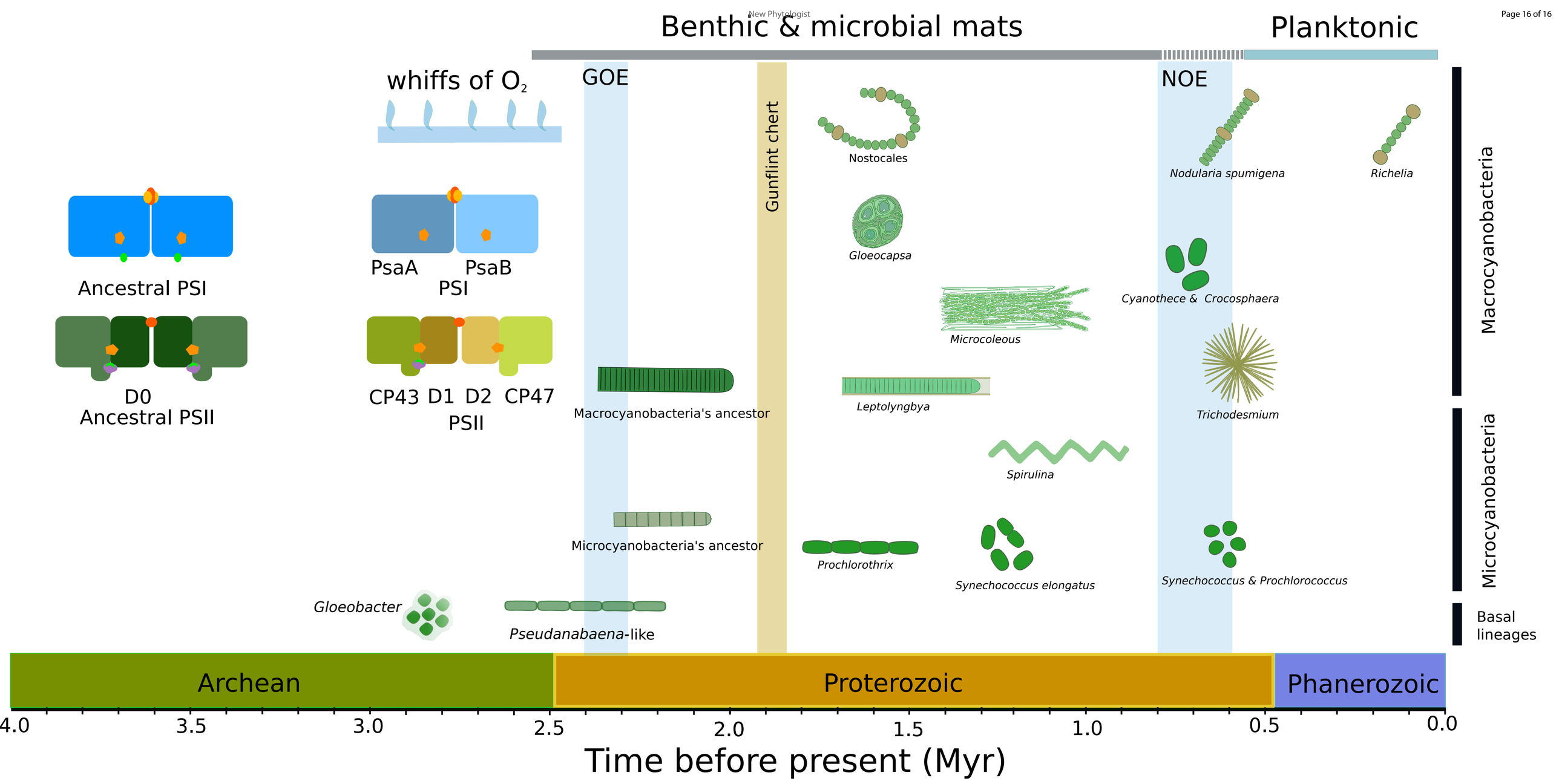
Benthic & microbial mats
Planktonic
whiffs of O_2
GOE
Gunflint chert
NOE
Nostocales
Nodularia spumigena
Richelia
Gloeocapsa
Cyanothece & Crocosphaera
Microcoleous
Trichodesmium
Leptolyngbya
Macrocyanobacteria's ancestor
Spirulina
Microcyanobacteria's ancestor
Prochlorothrix
Synechococcus elongatus
Synechococcus & Prochlorococcus
Gloeobacter
Pseudanabaena-like
Ancestral PSI
PsaA
PsaB
PSI
D0
Ancestral PSII
CP43 D1 D2 CP47
PSII
Macrocyanobacteria
Microcyanobacteria
Basal lineages
Archean
Proterozoic
Phanerozoic
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Time before present (Myr)